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Research Article

Variability Study of Biofortified Bread Wheat Genotypes for Grain Zinc and Iron Concentration, Yield and Yield Associated Traits at Khumaltar, Lalitpur, Nepal

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Abstract

Bread wheat (*Triticum aestivum* L.) is third major cereal crop of Nepal where cereal based foods represent the largest proportion of the daily diet. Lack of diverse food habit in the country is resulting micronutrient deficiency. This could be addressed by introducing biofortified bread wheat genotypes. This field research was conducted at Agriculture Botany Division, Khumaltar, Lalitpur to study the variability of biofortified bread wheat genotypes for grain zinc, iron, yield, yield attributes and identify high yielding genotypes with high grain zinc and iron concentration. 50 wheat genotypes (47 biofortified, 3 checks) were tested in alpha lattice design with two replications. Data on grain zinc, iron, yield and yield attributes were recorded and analyzed (α=5%). Genotypes differed significantly for the studied traits which provide an opportunity to improve the existing germplasms for targeted traits and environment. Grain yield was correlated positively with effective tiller, plant height, days to maturity, grains per spike and negatively with thousand grain weight. Grain zinc and iron concentration were significantly positively correlated but had positive non-significant relation with grain yield suggesting simultaneous improvement of both micronutrients without compromising grain yield is possible. Effective tiller and peduncle length can be used as selection criteria for high grain yield and micronutrient concentration respectively. Superior genotypes containing higher grain iron, zinc and yield can be used as parent in breeding for developing zinc and iron enriched varieties.

Keywords: Biofortified wheat; Grain Zinc; Grain Iron; Grain Yield; *Triticum aestivum*

Introduction

Bread wheat (*Triticum aestivum* L.) is a widely cultivated cereal grain of *Poaceae* family and is one of the major staple foods across the world. It is the second most important food crop worldwide after rice. World wheat harvest of the year 2016 was 749,460,077 tons from 220,107,551 ha land area (FAOSTAT, 2016). In Nepal wheat is third major cereal crop after rice and maize in terms of the area of production and productivity but second staple grain after rice in consumption. During 2016/17 wheat was grown in 735,850 ha land area with production of 1,879,191 metric tons and yielding 2,554 kg/ha (MoALMC, 2018). Micronutrient deficiency is a major problem affecting more than 2 billion people worldwide. In Nepal about 35% of women of reproductive age are found anemic during 2011 which increased upto 41% in 2016 and 50% children were also

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found anemic. More than 3/4 of apparently healthy women were found to be zinc deficient in Bhaktapur, Nepal (Chandyo *et al*., 2009). About 50% of children in Nepal suffer from stunting. The government has recognized the need for zinc supplements for stunted children. The economic cost of malnutrition mainly mineral & vitamin deficiency was estimated 2-3 % of GDP (US$ 250 to 375 million) every year in Nepal (World Bank, 2012). In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Bouis & Welch, 2010). Biofortification of cereal crops increases the micronutrient concentration in daily dietary intake and is the best prevention against micronutrient deficiencies. Wheat has been considered ideal for biofortification due to its significant role in ensuring food security (Singh & Velu, 2017).

CIMMYT initiated biofortification in wheat breeding in 2006 in collaboration with partners of HarvestPlus Program. The major objective of Biofortified Wheat Project is to develop nutritionally enhanced cultivars of common wheat (*Triticum aestivum* L.) to increase peoples' intake of Zinc and Iron (Velu, 2012). Screening of synthetic wheat, spelt wheat and wheat landraces with high micronutrient concentration were followed by crossing with high yielding, adopted bread wheat and selection for certain agronomic, disease resistance traits and micronutrient concentration. This resulted in incorporation of several novel alleles for micronutrient in high yielding adopted bread wheat varieties (Singh & Velu, 2017). Four biofortified bread wheat varieties Zincol 2016 in Pakistan, Zinc Shakti, WB02 and HPBW-01 in India has been released (Singh & Velu, 2017) but no biofortified bread wheat is released in Nepal till date. The biofortified wheat genotypes developed by CIMMYT were tested in this research to study the variation among the genotypes for grain zinc, grain iron, grain yield, yield attributes and to identify the best genotype with high grain iron and zinc concentration without compromising grain yield.

Materials and Methods

Plant Material

Forty-seven biofortified bread wheat genotypes with significantly improved Zn and Fe concentrations and desirable agronomic traits along with two commercial checks from CIMMYT and one local check WK 1204 from ABD, NARC (Table 1) were tested at the farm of Agriculture Botany Division, NARC, Khumaltar from 10th November 2016 to 20th May 2017. Geographically the research site is located in Kathmandu valley of mid hill region of Nepal at 27° 65' 18'' N latitude and 85° 32' 79'' E longitude at an altitude of 1365 masl. Field was laid in alpha lattice design with two replications, each replication consisting 5 blocks with 10 plots in each block. Each of the plots were of 6 m^2 area consisting of 6 rows of 4m length and 25cm row to row distance. All package of practice required to raise wheat were followed. Data recording in field condition was done for days to heading, days to maturity, number of effective tiller per m^2, plant height, spike length, peduncle length, grain yield, grain number per spike, grain weight per spike and thousand grain weight.

Micronutrient Sampling and Analysis

20 g of cleaned grain samples from each plot were analyzed in Wheat Quality Lab, Institute of Agriculture, Banaras Hindu University, Varanasi, India for micronutrient analysis with a bench-top, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model x-supreme8000, Oxford Instruments plc, Abingdon, UK) (Paltridge *et al*., 2012).

Genetic Parameters Estimation

Broad Sense Heritability

The broad sense heritability was calculated according to (D. S. Falconer, 1960)

$$Heritability\ (h^2) = \frac{Gennotypic\ variance\ (\sigma_g^2)}{Phenotypic\ variance\ (\sigma_p^2)} \tag{1}$$

Genotypic and phenotypic variance were obtained from analysis of variance table according to (Sing & El-Bizri, 1992)

$$\sigma_g^2 = = \frac{Genotypic\ mean\ sum\ of\ square - Error\ mean\ sum\ of\ square}{r} \tag{2}$$

$$\sigma_p^2 = Genotypic\ mean\ sum\ of\ square + Error\ mean\ sum\ of\ square \tag{3}$$

The heritability was categorized as 0-30% as low; 30-60% as moderate; 60% and above as high heritability (Robinson, Comstock, & Harvey, 1949).

Phenotypic and genotypic coefficient of variation

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated according to (Sing & El-Bizri, 1992)

$$\mathrm{GCV}(\%) = \frac{\sqrt{\sigma_g^2}}{X} * 100 \tag{4}$$

$$\mathrm{PCV}(\%) = \frac{\sqrt{\sigma_p^2}}{X} * 100 \tag{5}$$

Where,

$\sigma_g^2 = genotypic\ variance$

$\sigma_p^2 = phenotypic\ variance$

X= mean value of the trait

PCV and GCV were categorized as 0-10%;low, 10-20%;moderate and above 20%;high (Sivasubramanian & Menon, 1973)

Genetic Advance and Genetic Advance as Percentage of Mean

Genetic advance was calculated as per the following formula

$$GA = K * \sqrt{\sigma_p^2} * h^2 \quad (6)$$

Where,

K= Selection intensity (5%=2.06)

Genetic advance as % of mean was estimated according to (Hanson, Robinson, & Comstock, 1956)

$$\text{Genetic advance as \% of mean (GAM)} = \frac{\text{GA}}{\text{mean value of the trait}} * 100\% \quad (7)$$

Genetic advance ass % of mean is categorized as 0 to 10% low GAM, 10 to 20% moderate GAM and above 20% high GAM (Johnson, Robinson, & Comstock, 1955)

Statistical Analysis

The descriptive statistical analysis of variance (ANOVA) was estimated using R-Studio version: 3.3.0, correlation was estimated using META R software and UPGMA cluster was estimated using MINITAB 18. Statistical analysis was done at 5% level of significance.

Table 1: Pedigree of biofortified bread wheat genotypes

Source	Cross Name/ Pedigree
WK1204	WK1204 (LOCAL CHECK)
7HPYT402	BAJ #1
7HPYT403	KACHU #1
7HPYT404	CROC_1/ *Ae.squarrosa* (210)//INQALAB 91*2/KUKUNA/3/PBW343*2/KUKUNA
7HPYT405	T.DICOCCON CI9309/ *Ae.squarrosa* (409)//MUTUS/3/2*MUTUS
7HPYT406	DANPHE#1*2/3/*T.dicoccon* PI94625/*Ae.squarrosa*(372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO /5/WBLL1*2/KURUKU//HEILO
7HPYT407	DANPHE#1*2/3/*T.dicoccon* PI94625/*Ae.squarrosa* (372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO /5/WBLL1*2/KURUKU//HEILO
7HPYT408	DANPHE#1*2/3/*T.dicoccon* PI94625/*Ae.squarrosa*(372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO /5/WBLL1*2/KURUKU//HEILO
7HPYT409	CHONTE*2/SOLALA//2*BAJ #1
7HPYT410	FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO
7HPYT411	FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO
7HPYT412	FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO
7HPYT413	FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES/9/FRANCOLIN #1//WBLL1*2/BRAMBLING
7HPYT414	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/5/WAXWING/3/BL 1496/MILAN//PI 610750/4/FRNCLN/6/MUNAL/3/HUW234+LR34/PRINIA//PFAU/WEAVER
7HPYT415	KVZ/PPR47.89C//TACUPETOF2001*2/BRAMBLING/3/2*TACUPETOF2001*2/BRAMBLING /4/KACHU/5/KACHU #1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU
7HPYT416	KVZ/PPR47.89C//FRANCOLIN #1/3/2*PAURAQ/4/PBW343*2/KUKUNA*2//FRTL/PIFED/5/MUNAL #1
7HPYT417	HGO94.7.1.12/2*QUAIU #1//QUAIU #2/3/KINGBIRD #1//INQALAB 91*2/TUKURU/4/SUP152/BAJ #1
7HPYT418	HGO94.7.1.12/2*QUAIU#1//QUAIU#2/5/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES /6/MUCUY
7HPYT419	HGO94.7.1.12/2*QUAIU#1//QUAIU#2/5/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES /6/MUCUY
7HPYT420	CHIH95.2.6//WBLL1*2/KURUKU/3/WBLL1*2/KKTS/4/ND643/2*WBLL1/5/SAUAL/YANAC//SAUAL/6/WBLL1*2/BRAMBLING//VORB/FISCAL/3/BECARD
7HPYT421	CHIH95.2.6//WBLL1*2/KURUKU/3/WBLL1*2/KKTS/4/ND643/2*WBLL1/5/TACUPETO F2001/BRAMBLING*2//KACHU/6/KUTZ

Table 1: Pedigree of biofortified bread wheat genotypes

Source	**Cross Name/ Pedigree**
7HPYT422	*T.dicoccon*CI9309/*Ae.squarrosa*(409)//2*PANDORA/3/KINGBIRD#1//INQALAB 91*2/TUKURU/5/MUNAL/3/KIRITATI//PRL/2*PASTOR/4/MUNAL
7HPYT423	*T.dicoccon*CI9309/*Ae.squarrosa*(409)//2*PANDORA/5/WAXWING/3/BL1496/MILAN//PI 610750/4/FRNCLN/6/KACHU/BECARD//WBLL1*2/BRAMBLING
7HPYT424	HGO94.7.1.12/2*QUAIU#1//WAXBI/5/WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX
7HPYT425	COAH90.26.31/4/2*BL2064//SW89-5124*2/FASAN/3/TILHI/5/UP2338*2/KKTS*2//YANAC /6/MUTUS/AKURI
7HPYT426	COAH90.26.31/4/2*BL2064//SW89-5124*2/FASAN/3/TILHI/5/UP2338*2/KKTS*2//YANAC /6/MUTUS/AKURI
7HPYT427	CROC_1/*Ae.squarrosa* (210)//INQALAB 91*2/KUKUNA/3/PBW343*2/KUKUNA/5/SAUAL/3/C80.1 /3*BATAVIA//2*WBLL1/4/SITE/MO//PASTOR/3/TILHI/6/SAUAL #1/KACHU
7HPYT428	UC1113-GPCB1/3/TACUPETO F2001/BRAMBLING*2//KACHU/4/TACUPETO F2001/BRAMBLING //KACHU
7HPYT429	CHONTE*2/SOLALA/5/GARZA/BOY// *Ae.squarrosa* (467)/3/T.DICOCCON PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/*T.dicoccon*PI94625/*Ae.squarrosa*(372)//3*PASTOR/6/ATTILA*2/PBW65//PIHA /3/ATTILA/2*PASTOR
7HPYT430	QUAIU #1/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/QUAIU #2/5/BORL14
7HPYT431	DANPHE #1*2/SOLALA/3/ATTILA*2/PBW65//MURGA
7HPYT432	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/4/COAH90.26.31//KIRITATI/WBLL1 /3/KIRITATI/2*WBLL1
7HPYT433	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/3/PBW343*2/KUKUNA*2//FRTL/PIFED
7HPYT434	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/3/PBW343*2/KUKUNA*2//FRTL/PIFED
7HPYT435	*T.dicoccon* CI9309/ *Ae.squarrosa* (409)//MUTUS/3/2*MUTUS/5/ *T.dicoccon* PI94624/ *Ae.squarrosa* (409)//BCN/3/WAXWING/4/2*FRNCLN
7HPYT436	*T.dicoccon*CI9309/ *Ae.squarrosa* (409)//MUTUS/3/2*MUTUS/4/FRET2/TUKURU//FRET2*2/3/*T.spelta* PI348530
7HPYT437	*T.dicoccon*CI9309/ *Ae.squarrosa* (409)//MUTUS/3/2*MUTUS/4/FRET2/TUKURU//FRET2*2/3/*T.spelta* PI348530
7HPYT438	*T.dicoccon*CI9309/*Ae.squarrosa*(409)//MUTUS/3/2*MUTUS/5/PFAU/WEAVER*2/4/BOW/NKT //CBRD/3/CBRD
7HPYT439	*T.dicoccon*PI94624/*Ae.squarrosa*(409)//BCN/3/WAXWING/4/2*FRNCLN/5/VILLAJUAREZ F2009/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/WBLL1*2/BRAMBLING
7HPYT440	CHIH95.2.6/4/BABAX/LR42//BABAX*2/3/SHAMA/5/2*BABAX/LR42//BABAX*2/3/TUKURU/6/KFA/2*KACHU
7HPYT441	HGO94.7.1.12/2*QUAIU #1/3/VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING
7HPYT442	HGO94.7.1.12//WBLL1*2/KUKUNA/3/WBLL1*2/KURUKU/4/PBW343*2/KUKUNA*2//FRTL/PIFED
7HPYT443	COAH90.26.31//KIRITATI/WBLL1/3/KIRITATI/2*WBLL1/7/OASIS/SKAUZ//4*BCN/3/2*PASTOR/4/*T.spelta* PI348449/5/BACEU #1/6/WBLL1*2/CHAPIO
7HPYT444	VILLA JUAREZ F2009/3/*T.dicoccon*PI94625/*Ae.squarrosa*(372)//3*PASTOR/4/WBLL1*2/ BRAMBLING/5/QUAIU#1/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/QUAIU #2
7HPYT445	VILLA JUAREZ F2009/3/*T.dicoccon*PI94625/*Ae.squarrosa*(372)//3*PASTOR/4/WBLL1*2/ BRAMBLING/5/QUAIU#1/3/*T.dicoccon*PI94625/*Ae.squarrosa* (372)//3*PASTOR/4/QUAIU #2
7HPYT446	VILLA JUAREZ F2009/3/ *T.dicoccon*PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/WBLL1*2/BRAMBLING/5/QUAIU #1/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/QUAIU #2
7HPYT447	VILLA JUAREZ F2009/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/WBLL1*2/BRAMBLING/7/OASIS/SKAUZ//4*BCN/3/2*PASTOR/4/*T.spelta* PI348449/5/BACEU #1/6/WBLL1*2/CHAPIO
7HPYT448	VILLA JUAREZ F2009/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/WBLL1*2/BRAMBLING/5/BAJ #1/AKURI
7HPYT449	VILLA JUAREZ F2009/3/ *T.dicoccon* PI94625/ *Ae.squarrosa* (372)//3*PASTOR/4/WBLL1*2/BRAMBLING/5/ATTILA*2/PBW65//MUU #1/3/FRANCOLIN #1
7HPYT450	68.111/RGB-U//WARD/3/ *Ae.squarrosa* (321)/4/INQALAB 91*2/KUKUNA/5/PBW343*2/KUKUNA/6/MUCUY

Table 2: Mean performance of biofortified bread wheat genotypes for grain yield, yield attributes and micronutrients

	PH (cm)	SL (cm)	PL (cm)	DH	DM	TN	GPS	GWPS (gm)	TGW (gm)	FeC (ppm)	ZnC (ppm	GY (t/ha)	FeY (kg/ha)	ZnY (kg/ha)
Grand Mean	104	10.61	16.42	126	174	311	58.54	3.07	52.9	39.80	42.50	6.5	259.7	277.6
Range	93-113	9.1-11.9	9.9-25.5	115-134	163-183	162-424	42-76	2.4-3.8	44.8-66.2	32.6-49.9	33-53.5	3.7-8.1	138-365	126-407
CV	4.11	6.71	9.08	1.73	1.15	12.49	10.6	8.88	6.56	9.89	10.47	9.52	12.47	12.8
LSD	8.62	1.43	3.01	4.42	4.04	78.49	12.53	0.55	7.01	7.95	8.99	1.26	65.45	71.82
F-value	2.17	2.11	10.90	6.56	11.21	3.43	2.26	2.68	2.97	2.08	2.57	3.56	3.48	3.92
F test	**	**	***	***	***	***	**	***	***	**	**	***	***	***
SEM	0.542	0.09	0.36	0.46	0.52	6.06	0.79	0.04	0.49	0.49	0.62	100.7	4.97	5.85

Table 3: Genotypic (above diagonal) and phenotypic (below diagonal) correlation grain yield, yield attributes and micronutrients

	DTH	DTM	PH	PL	TN	GPS	GWPS	TGW	GY	FeC	ZnC	FeY	ZnY
DTH	1	0.84***	-0.123	-0.78***	0.321*	0.436*	0.14	-0.413**	0.340*	-0.443**	-0.002	0.033	0.272
DTM	0.80***	1	-0.177	-0.61***	0.423**	0.500***	0.142	-0.49***	0.367**	-0.469**	-0.135	0.048	0.252
PH	-0.059	-0.044	1	0.365***	0.279*	0.404**	0.483***	0.051	0.529***	0.293*	0.700***	0.543***	0.786***
PL	-0.7***	-0.57***	0.219	1	-0.077	-0.44***	-0.241	0.258	-0.118	0.607***	0.307*	0.222	0.086
TN	0.197	0.354*	0.156	-0.06	1	0.259	-0.49***	-0.99***	0.766***	-0.137	-0.081	0.575***	0.597***
GNPS	0.378**	0.399**	0.188	-0.391**	0.059	1	0.708***	-0.414**	0.560***	-0.51***	0.064	0.131	0.446***
GWPS	0.154	0.144	0.262	-0.239	-0.353*	0.730***	1	0.334*	0.174	-0.213	0.139	0.008	0.238
TGW	-0.332*	-0.378**	0.042	0.243	-0.55***	-0.462**	0.263	1	-0.48***	0.492***	0.218	-0.126	-0.221
GY	0.252	0.349*	0.424**	-0.09	0.658***	0.274	0.106	-0.259	1	0.148	0.061	0.851***	0.787***
FeC	-0.279*	-0.301*	0.108	0.398**	-0.1	-0.275	-0.024	0.377**	0.02	1	0.806***	0.621***	0.591***
ZnC	-0.159	-0.174	0.246	0.204	0.076	-0.048	0.075	0.163	0.028	0.523***	1	0.488***	0.651***
FeY	0.076	0.163	0.39***	0.1310	0.48***	0.0368	0.0555	0.0209	0.796***	0.611***	0.332**	1	0.943***
ZnY	0.135	0.253	0.44***	0.023	0.558***	0.164	0.115	-0.091	0.748***	0.313**	0.670***	0.791***	1

DTH: Days to heading; DTM: Days to maturity; PH: Plant height; SL: Spike Length; PL: Peduncle length; TN: Number of effective tiller per m2; GPS: Grain number per spike; GWPS: Grain weight per spike; TGW: Thousand grain weight; GY: Grain yield; FeC: Grain iron concentration; ZnC: Grain zinc concentration; FeY: Grain iron yield; ZnY: Grain zinc yield; Significant correlations are denoted by * for $p < .05$, ** for $p < .01$, and *** for $p < .001$

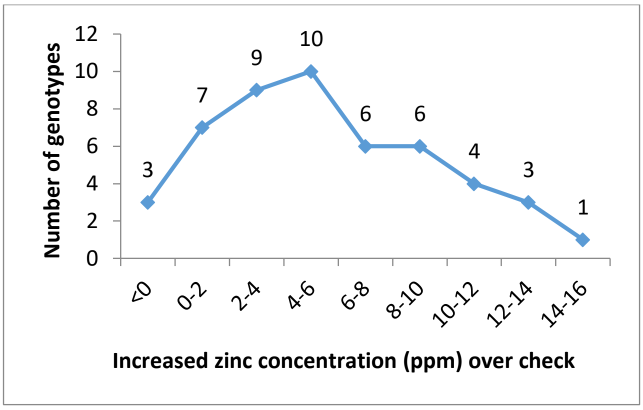

Fig 1. Grain Zinc concentration of biofortified bread wheat genotypes over local check WK 1204

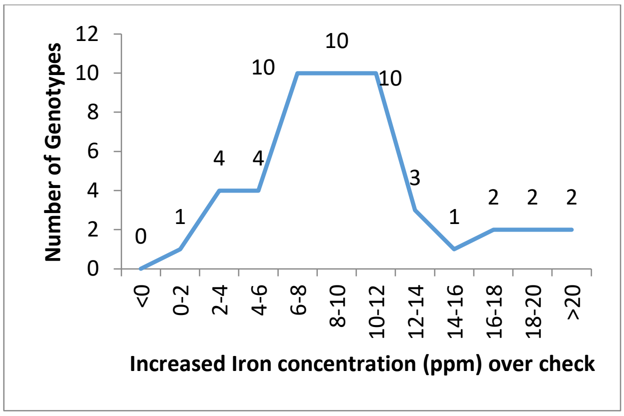

Fig 2. Grain Iron concentration of biofortified bread wheat genotypes over local check WK 1204

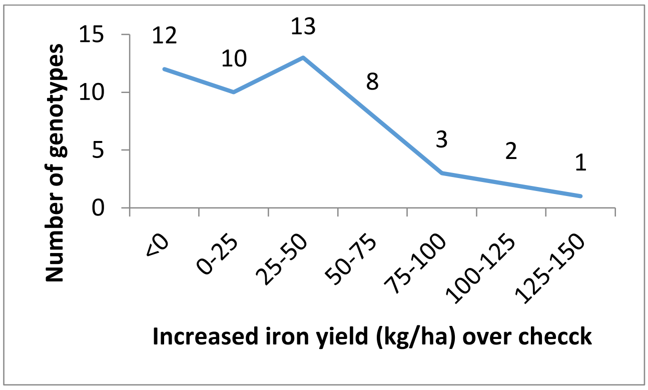

Fig 3. Iron yield of biofortified bread wheat genotypes over local check WK 1204

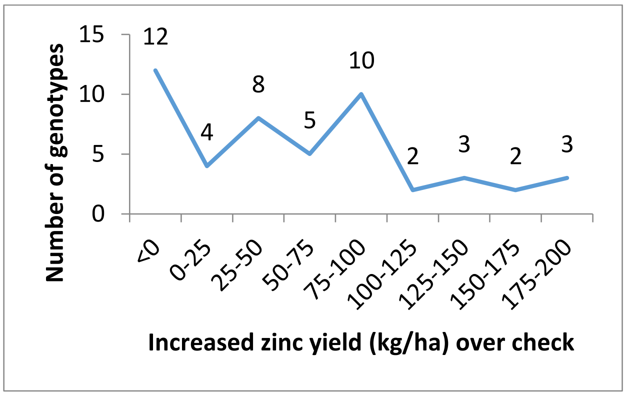

Fig 4. Zinc yield of biofortified bread wheat genotypes over local check WK 1204

Result and Discussion

Mean Performance

Genotypes differed significantly for all the studied traits (Table 2). The genotypes differed significantly for days to heading with the range of 115 days to 134 days and days to maturity with the range of 163 to 183 days. Number of effective tiller ranged from 162 to 424 per m^2. Plant height ranged from 93 cm to 113 cm, peduncle length ranged from 9.9 cm to 25.5 cm. and spike length ranged from 9.1 cm. to 11.9 cm. The number of grain per spike varied significantly with the range of 42 to 76, grain weight per spike with the range of 2.44gm to 3.84gm, thousand grain weight with the range from 44.8gm to 66.2 gm. The genotypes significantly varied on grain iron concentration ranging from 32.59 ppm to 49.97 ppm and grain zinc concentration from 33.0 ppm to 53.46 ppm. Among all the tested genotypes 94% genotypes had grain zinc concentration higher than the local check WK 1204 (Fig. 1) and 100% genotypes had grain iron concentration higher than the local check WK 1204 (Fig. 2). Grain iron yield of tested genotypes ranged from 138.3 kg/ha to 365.7 kg/ha and Zinc yield ranged from 126.4 kg/ha to 407.0 kg/ha. Among all the tested genotypes 76% genotypes had zinc yield and grain iron yield more than local check WK 1204 (Fig. 3, Fig. 4).

The genotypic and phenotypic correlations between grain zinc and iron concentration were significantly positive (Table 3) which means that the grain zinc and iron concentration can be simultaneously improved through selection. Similar association was reported between grain iron and zinc concentration by Velu *et al.*, (2012); Chatrath *et al.*, (2018); Liu *et al.*, (2014); Monasterio & Graham, (2000).The grain iron yield was significantly positively correlated with grain yield and similar result was reported by Velu *et al.*, (2012). The grain zinc yield was significantly positively correlated with grain yield and similar result was obtained by Velu *et al.*, (2012). The positive relation between yields of grain iron and grain zinc with grain yield suggests that breeding for high zinc and iron yield do not have any negative effect in grain yield potential Velu *et al.*, (2012). The grain yield had significant positive genotypic correlation with days to heading and maturity, plant height, number of effective tiller per m^2, number of grain per spike, grain iron concentration (Table 3). The strongest genotypic correlation of grain yield was found with number of effective tiller per m^2 (0.766***) and plant height (0.529***). Similarly, strong phenotypic correlation of grain yield was found with effective number of tillers per m^2 (0.658***) and plant height (0.424**). According to Joshi *et al.*, 2008, the number of effective tiller and grains per spike are the most important traits for grain yield in wheat. Waqar-Ul-Haq *et al*., (2010) also reported correlation between effective tiller number per m^2, number of grain per spike, number of spike per plant with grain yield was found significantly positive. From this research, genotypic correlation between grain yield and thousand grain weight was found significant negative (-0.48***) which reveals genotypes having higher yields has higher number of grains. Negative correlation between thousand grain weight and grain yield was also reported by Mohammadi *et al*., (2012) ; Khan, Azam, & Ali, (2010) but significantly positive correlation between thousand grain weight and grain yield was reported by Joshi *et al*., (2008); Kashif & Khaliq, (2004). Based on the mean performance, correlation and regression analysis grain yield could be increased by selection based on number of effective tiller per m^2, plant height, number of grains per spike, grain weight per spike.

Genetic Parameters

Heritable genetic variation in targeted traits has great importance in crop genetic improvement. Hence the available genetic variation, heritability and expected genetic gain are useful to develop better breeding strategies (Jalata, Ayana, & Zeleke, 2011). Grain yield is a polygenic trait having low heritability and often affected by environment, so the selection for grain yield is difficult (Riaz & Chowdhry, 2003). The genotypic coefficient of variation and heritability estimates provide the scope of genetic advance to be expected through phenotypic selection. The value of GCV was high for peduncle length (20.2), moderate for effective tiller per m^2 (13.8) and grain yield per ha (10.6) and low for other remaining traits (Table 4). The PCV was found higher than GCV for each traits but the difference between PCV and GCV is less which shows less environmental effects on the traits expression since PCV is result of GCV and ECV. If proportion of GCV of studied trait exceeds environmental effect at higher level, selection of genotypes is recommendable for improvement further. Similar finding was reported by Jalata, Ayana, & Zeleke, (2011); Tsegaye, Dessalegn, Dessalegn, & Share, (2012). The character with high heritability but low genetic advance is governed by non-additive gene action, whereas the character with high heritability and high genetic advance is governed by additive gene action (Eid, 2009). Peduncle length was found with high heritability (83.2%) and high genetic advance (38.0%) and this trait is found to have additive gene action (Table 4). Also, peduncle length had highly significant positive genotypic correlation with grain number per spike, grain iron concentration and grain zinc concentration but it had highly significant negative genotypic correlation with days to heading and maturity. So the selection based on peduncle length would be effective on improvement of grain zinc and iron concentration and earliness of variety. Moderate heritability (54.9%) and high genetic advance (20.2%) is found for the number of effective tiller per m^2 which is strongly correlated with grain yield. Selection based on tiller number per m^2 would be effective on improving grain yield. Ali *et al*., (2008) reported high heritability followed by high genetic gain for number of effective tiller per m^2. Moderate heritability followed by moderate genetic gain for grain yield, grain zinc concentration, grain number per spike, grain weight per spike showed moderate scope for improvement of these traits. High heritability for days to heading, days to maturity, moderate heritability for plant height, spike length, thousand grain weight and grain iron concentration with low genetic advance indicates non-additive gene effects and limited scope of improvement of these traits through selection (Jalata *et al*., 2011).

Cluster Analysis

The cluster analysis based on days to heading and maturity, plant height, peduncle length, number of effective tiller per m^2, number of grain and its weight per spike, thousand grain weight, grain yield, grain iron and zinc concentration divided the genotypes in 5 different clusters (Fig.5). Cluster I consisted of 29 genotypes representing the genotypes with moderate days to heading, days to maturity, plant height, number of effective tiller per m^2, grain weight per spike, grain number per spike, thousand grain weight, grain yield, grain iron concentration and grain zinc concentration. Cluster II consists of 5 genotypes with highest yield and other yield attributing traits. These genotypes (7HPYT402, 7HPYT426, 7HPYT405, 7HPYT410 and 7HPYT429) could be selected for gaining highest yield with high grain zinc and iron concentration. This cluster is superior for number of effective tillers per m^2, grain number and its weight per spike and grain yield. The thousand grain weight is lowest for the genotypes of cluster II. Cluster III with 13 genotypes (7HPYT403, 7HPYT404, 7HPYT438, 7HPYT407, 7HPYT420, 7HPYT432, 7HPYT439, 7HPYT406, 7HPYT428, 7HPYT422, 7HPYT436, 7HPYT413 and 7HPYT418) could be selected for highest grain iron and zinc concentration with average yield. This cluster is very important for the genotypes with highest grain zinc and grain iron concentration and longest peduncle length. The cluster IV consisted of 1 genotype 7HPYT419 with earliest heading and maturity, very short in plant height, highest per spike and 1000 grain weight but has lowest number of effective tillers per m^2 lowest grain iron concentration. This genotype could be used in breeding for early maturity, larger grains. The cluster V consisted of 2 genotypes 7HPYT431 and 7HPYT449 with very late in heading and maturity, least peduncle length, and grain iron concentration. The genotypes of cluster I and cluster II are found to be closely related whereas the genotypes of cluster II and cluster IV are most distantly related (Table 5). According to Rahim *et al.,* (2010) the hybridization between distantly related genotypes produce high yielding hybrids, so the genotypes of cluster II and cluster IV can be used for hybridization program for transgressive character

Table 4. Estimation of heritability, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and genetic advance as percentage of mean (GAM)

Traits	Genotypic variance	Phenotypic variance	Environmental variance	Broad Sense Heritability (%)	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Genetic Advance as Percentage of Mean (%)
DH	13.32	18.11	4.79	73.55	3.37	2.89	5.10
DM	20.48	24.49	4.01	83.62	2.84	2.60	4.89
PH	10.67	28.89	18.23	36.91	5.18	3.15	3.94
SL	0.28	0.79	0.51	35.75	8.38	5.01	6.17
PL	11.02	13.25	2.23	83.19	22.16	20.22	37.98
TN	1838.9	3349.7	1510.8	54.9	18.61	13.79	21.05
GPS	24.27	62.80	38.53	38.65	13.54	8.42	10.78
GWPS	0.06	0.14	0.07	45.63	12.05	8.14	11.32
TGW	11.72	23.77	12.06	49.29	9.21	6.47	9.36
GY	494842	881699	386857	56.12	14.38	10.77	16.62
FeC	15.56	35.38	19.82	43.99	14.00	9.28	12.68
ZnC	8.40	23.90	15.50	35.15	12.28	7.28	8.89

Table 5: Distance between different cluster centroids of biofortified bread wheat genotypes

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	0				
Cluster2	644.97	0			
Cluster3	635.96	1280.91	0		
Cluster4	2291.49	2936.38	1655.57	0	
Cluster5	1217.24	1862.18	581.45	1074.69	0

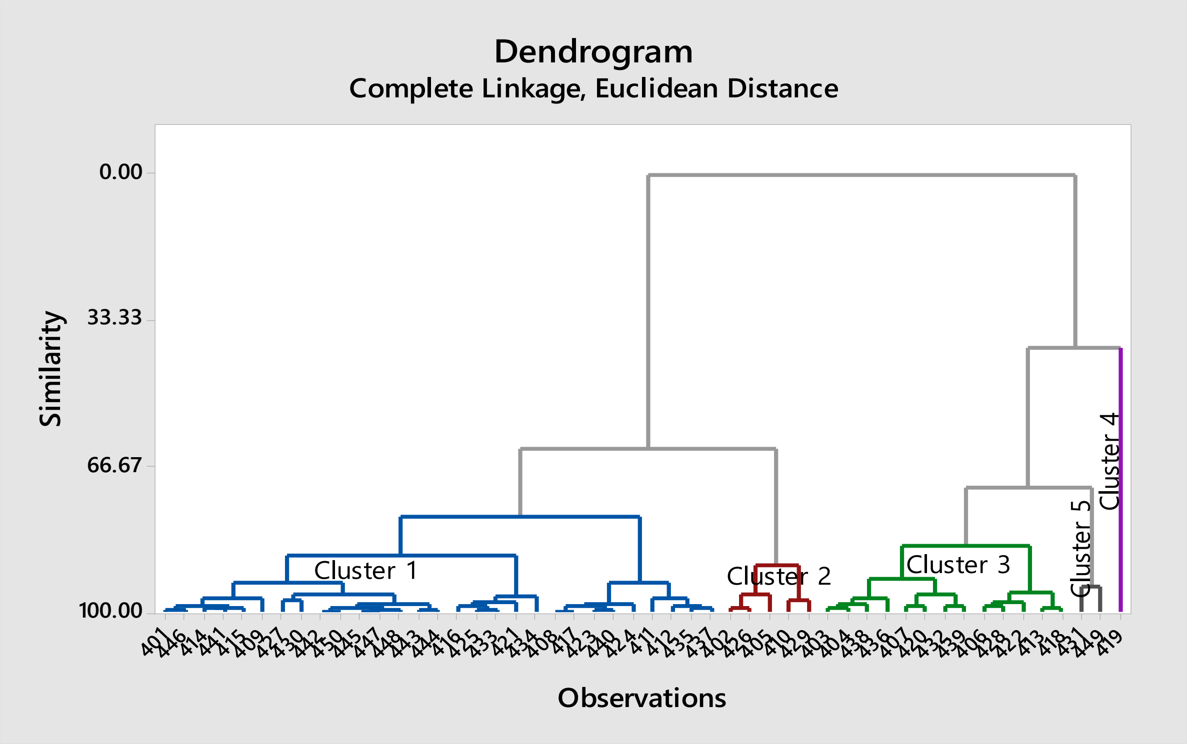

Fig. 5: Unweighted pair group method with arithmetic mean (UPGMA) clustering of 50 biofortified bread wheat genotypes

Table 6: The average of traits for each cluster obtained from UPGMA cluster analysis

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Grand Centroid
Days to heading	126.8	127.66	124.29	121.59	131.35	126.31
Days to maturity	175.15	175.96	171.2	164.6	179.01	174.15
Plant height	103.9	104.94	103.51	97.59	101.59	103.68
Peduncle length	16.71	14.07	17.64	15.13	11.02	16.43
Number of effective tiller per m^2	319.55	340.67	293.56	207.5	277.93	311
Grain number per spike	58.42	60.8	58.22	54.9	58.52	58.54
Grain weight per spike	3.06	3.15	3.07	3.17	3.11	3.08
Thousand grain weight	52.8	51.73	53.09	59	53.19	52.91
Grain yield	6727.2	7371.8	6091.8	4438.5	5510.7	6532
Grain iron concentration	39.48	40.36	40.75	39.88	37.11	39.81
Grain zinc concentration	42.28	42.12	43.29	40.74	42.34	42.5

Conclusion

Large variation was found among genotypes for the studied traits which provide an opportunity to improve the existing germplasms for targeted traits and environment. Biofortified wheat lines were found to be superior for grain zinc and iron content than non-biofortified wheat varieties and also high yielding as compared to non-biofortified wheat varieties including WK1204. Genotypes identified for high grain zinc and iron concentration with high grain yield could be used for breeding to develop micronutrient rich wheat. The traits namely plant height and peduncle length may be considered as indirect selection criteria for improving grain Fe and Zn concentration. Indirect selection for grain yield could be carried out based on the number of effective tiller per m^2. Simultaneous improvement of both zinc and iron concentration without compromising grain yield is possible through wheat breeding.

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